Edn1fl/fl;Foxg1-Cre embryos. Our findings thus establish that in mice, the ectoderm is the main source of Edn1 driving Ednra-dependent facial development. This work was funded by NIDCR DE014181.

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Program/Abstract #432
Nkx2.5 regulates hand2 expression in the zebrafish pharyngeal arches via a conserved enhancer
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Craniofacial development is mediated by complex and conserved pathways that regulate neural crest cell (NCC) induction, migration and development. NCCs arise from the neural folds and migrate ventrally into the pharyngeal arches before forming the craniofacial skeleton. A critical step in this development involves patterning the NCC-derived mesenchyme within the pharyngeal arches. Recent work in both mouse and zebrafish has shown that the bHLH factor Hand2 is crucial in patterning the ventral arch. In mouse, an enhancer has been identified that is sufficient to drive transgene expression within the arches in a Hand2-specific fashion. To better understand how ventral Hand2 expression is established, we used comparative genomics to identify a region upstream of the zebrafish hand2 locus that shows a 73.5% sequence similarity to the known mouse Hand2 arch specific enhancer and contains a number of conserved potential transcription factor binding sites. This putative enhancer was used to create hand2-mCherry transgenic lines in which mCherry is expressed in a Hand2-specific pattern in the arches. Among candidate regulatory factors, Nkx2.5 showed the strongest enhancer activation in luciferase assays. Deletion of the Nkx2.5 binding site in the enhancer led to decreased luciferase expression, supporting its potential role in Hand2 regulation. Morpholino knockdown of nkx2.5 resulted in decreased hand2 mRNA in the arches as well as later facial cartilage defects similar to those observed in hand2 mutants. Our findings thus suggest that Nkx2.5 is involved in inducing ventral hand2 expression in the arches, which then establishes a ventral-specific developmental program. This work was supported by NIDCR (DE018899).

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Program/Abstract #433
The role of Fox genes in craniofacial development in zebrafish
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Fox genes encode a family of forkhead box transcription factors that have been shown to play multiple roles during development. Specifically, foxc1 and foxc2 in mice have been shown to be essential for regulating proliferation and differentiation of the mesenchyme giving rise to the axial and skull skeleton. This function seems to be, at least partially, under the control of the Sonic hedgehog signaling pathway. There is also evidence that during calvaria development, foxc1 is regulated through Bmp and Fgf signaling cascades. However, not much is known about the involvement and regulation of fox genes in the development of the anterior neural-crest-derived craniofacial skeleton. Work in mouse mutants has lead to a proposal of a model in which Fox genes form a complex expression code, much like the Dlx genes, throughout the craniofacial primordia. This model is yet to be tested. In this study, we describe the expression of foxc1a, foxc1b and foxd1 during craniofacial development in zebrafish. We find these genes to be expressed in very specific domains of the neural crest cell populated pharyngeal arches at 36hpf. Using transgenic approaches we show that the expression of these genes is up-regulated by Sonic hedgehog signaling and down-regulated by Fgf signaling. This suggests an evolutionarily conserved regulation of Fox genes between different taxa. We also demonstrate, using morpholino and transgenic approaches, that Fox genes are involved in patterning of specific skeletal elements derived from the pharyngeal arches. Specifically, our work shows that foxc1b might be involved in the specification of cartilage versus bone in the dorsal hyoid arch.

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Program/Abstract #434
Missing intramembranous bones in the skull via knockdown of SHH and BMP
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A series of small flat intramembranous bones is situated within the sclera of the chicken eye. Each bone plate is induced by a separate epithelial–mesenchymal induction mechanism. We initially investigated the induction and patterning of this ring of bones with a candidate gene approach and real time PCR. More recently, we have used embryonic manipulation, and applied cyclopamine and noggin-soaked beads to inhibit the Hedgehog and Bone Morphogenetic Protein families. Both result in preventing ossicle formation but the mechanisms of each are different. Furthermore, we identify shh and ptc-1 mRNA and proteins first in the conjunctival epithelium and later in the scleral mesenchyme, suggesting an autocrine short-range followed by a paracrine long-range signaling mechanism for this morphogen. In contrast knockdown of BMPs prevents the condensation within the mesenchyme from forming, and affects growth of neighboring bones. We are exploring the potential interplay between these two families, which will be discussed together with our current microarray analysis of the induction of this neural crest-derived intramembranous tissue.

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Program/Abstract #435
Yin–Yang1 is required in the epiblast during mammalian gastrulation
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One of the many ways that the Polycomb Group Gene, Yin–Yang1 (Yy1), is thought to regulate gene expression is through direct binding to DNA elements found in promoters or enhancers of target loci. In order to further define the role of YY1 during gastrulation and identify in vivo targets we deleted Yy1 specifically in the epiblast of mouse embryos. Yy1 conditional knockout (cKO) embryos initiate gastrulation and generate all three primary germ layers. However, the mesoderm and endoderm that are specified have abnormal gene expression of many critical developmental factors including Brachyury, Fgf8, Nodal, Pitx2, Cdh1 and Oct4. Yy1 mutants fail to undergo proper morphogenic movements typical of gastrulation and do not form a node, notochord or appropriately structured allantois. Our results reveal critical requirements of YY1 in several important developmental processes, including epithelial to mesenchymal transition and expression of the OCT4 pluripotency complex. Here